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Short communication

Decolorization/deodorization of zein via activated carbons and molecular sieves☆

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ABSTRACT

Commercially available corn zeins from co-products of the corn ethanol industry possess yellow color and off-odors that deter their usage in food, medical, pharmaceutical and cosmetic industries. A pilot-scale process (patent pending) was developed to purify those products. Our objective is to investigate a series of activated carbons and zeolites, clay-based particles acting as molecular sieves, as potential column media for that process. A batch process was used to evaluate each of five different activated carbons and zeolites to determine the adsorption characteristics of protein and the color and off-odor components. Adsorptive properties of those media were performed by spectral analyses at wavelengths 280 nm for the protein component and 325 nm for the contaminants. Statistical evaluation of the batch adsorption data from each series demonstrated that activated carbon generated from coconut hulls and zeolite 5 A (5 angstrom pore size) adsorbed the least amount of protein relative to contaminant adsorptions. Our selection of column media for the pilot-scale process was based on these findings.

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1. Introduction

Corn zein, a co-product of the ethanol industry, is a predominant corn protein that has numerous potential industrial uses in paints, inks, paper and board coatings, packaging of food products, microencapsulating agents, adhesives in laminated wood products and a base for chewing gum. A major deterrent for its uses in many products is its yellow color and off-odor. Sessa et al. (2003) explored a variety of methods for decolorizing zein including solvent partitioning, ultra-filtration/diafiltration on a tangential flow system, column chromatography on Sephadex LH60, subcritical propane and supercritical CO_2 extractions with alcohol entrainers and batch treatment with activated carbon. Statistical treatment of those decolorization methods demonstrated the superiority of the activated carbon treatment.

Activated carbon treatments to purify yellow zein have been used for many decades (Mason and Palmer, 1934; Swallen, 1938; Pearce, 1941; Starling et al., 1951; McInnis and Tang, 2003). Activated carbons are non-specific adsorbents that not only bind the color components of zein but also the protein components, whereby, the recovery of a decolorized zein product is greatly diminished. Recently, Sessa and Palmquist (2008) used a Freundlich isotherm model to evaluate the adsorption of zein protein and its color/odor components onto an activated carbon. In that publication the major contributors of zein odor were attributed to diferuloylputrescine. The measurement of protein adsorption by activated carbon was based on ultraviolet light absorbance at wavelength 280 nm and the odor component adsorption based on ultraviolet light absorbance at wavelength 325 nm. The removal of odor component was found to coincide with the removal of xanthophylls that cause yellow coloration.

A patent application was filed by Sessa (2007) for decolorizing and deodorizing corn zein products. The objective of this manuscript is to evaluate a series of activated carbons generated from different media and a series of zeolites with different pore sizes for potential use as a column media for that patent. Because these media bind protein as well as the corn zein impurities, the selection of column media was based on an activated carbon and a zeolite that can be used to achieve the highest yield of decolorized/deodorized zein product.

2. Materials and methods

2.1. Materials

Yellow zein (F-4000) with proximate analysis, dry basis: 93.09% crude protein (Dumas N × 6.25), 5.26% crude fat, 0.04% crude fiber, 0.05% ash, was purchased from Freeman Industries LLC, Tuckahoe,

☆ Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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NY. Activated carbons were provided by Norit America Inc., Marshall, TX: (1) Sonoborit B3, extruded; (2) GCN48 (now GCA48), coconut hull; (3) Norit GAC 830+,coal (acid-washed); (4) Norit CGran, wood; (5) Darco 12 × 20, lignite (acid-washed).

Molecular sieves: (1) Zeobest (natural clinoptilolite), 0.55–0.65 mm, from Northern Filter Media, Muscatine, IA; (2) Zeolite 3A; (3) Zeolite 4A; (4) Zeolite 5A; (5) Zeolite 13X purchased from Sigma–Aldrich Corp., St. Louis, MO. All zeolites are 8–12 mesh beads.

2.2. Experimental procedure

The activated carbon granules were each sieved through a number 30 screen to remove fine carbon powder. That portion, retained on the screen was washed three times with excess of 70% aqueous ethanol (w/w) to further remove powdered carbon adhering to the surface of the carbon granules. Likewise, the molecular sieves were each similarly hydrated and washed with excesses of 70% ethanol. The drained, washed granules, after evaporation of ethanol, were dried overnight in a hot air over at 105 °C. Ten grams of each dried granular adsorbent were measured into ten 250 mL Erlenmeyer flasks. A stock solution containing 0.4% yellow zein was prepared in 70% ethanol, where 100 mL increments were added to each flask. The flasks were then sealed shut with a rubber stopper. Individual flasks were shaken at 50 rpm for 24 h, the equilibrium time previously determined by Sessa and Palmquist (2008), at either 25 °C or 55 °C. Shaking was performed on an Innova 4000 Incubator Shaker, New Brunswick Scientific Co. Inc., Edison, NJ. After 24 h, shaking was stopped; the granular adsorbents settled and the liquid was decanted into a graduated cylinder to measure volume recovered. Measuring the decanted volume ensures an accurate concentration calculation.

Ultraviolet absorbances of each liquid fraction were measured at 280 nm, representative of protein, and 325 nm, representative of diferuloylputrescine, a contributor to off odor of zein (Sessa and Palmquist, 2008). Percentages of protein and odor component adsorbed were calculated at each wavelength relative to the control sample absorbances run with no adsorbent. Three replicates for each set consisting of 10 g adsorbent were run for each of the ten granular adsorbents.

2.3. Experimental design

A completely randomized design (CRD) was used to examine differences in five activated carbons and five molecular sieves. Experiments were performed at two different temperatures, 25 °C and 55 °C, with three replicates for percent protein and percent odorant adsorptions.

2.4. Statistical analysis

For the activated carbon series, single factor analyses of variance (ANOVA) models were used to analyze sieve differences separately at each temperature for percent protein and percent odor adsorbed.

For the molecular sieve adsorbents, two factor ANOVA models were used to analyze sieve and temperature differences for percent protein and odor adsorptions.

Data were checked for transformation necessity prior to analysis to satisfy ANOVA assumptions by using a Levene's test for homogeneity of variance All analyses were performed on a transformed data where necessary, but raw data means and standard deviations are presented for ease of interpretation.

If an *F*-test statistic was obtained from an ANOVA at $p \leq 0.05$, effects were considered to be significant. Differences of least square means at $p \leq 0.05$ were used as the multiple comparison test when more than two treatment means were being compared for mean adsorbent differences when a significant *F*-test from the ANOVA was obtained.

All analyses were performed using SAS PC Windows Version 9.1.3 software.

3. Results and discussion

3.1. Results with activated carbons

No transformations of data for percent of protein adsorbed and percent of odor adsorbed were needed when differences between activated carbons were analyzed separately for each temperature. Significant activated carbon differences in mean % protein and % odor adsorbances were found from all four ANOVAs (* each had $p \leq 0.0001$).

From data in Table 1, Darco 12 × 20 carbon consistently has the highest % protein and odor/color adsorptions over both temperatures. The GCN 48 (now GCA 48) carbon consistently has significantly less % protein and % odor/color adsorptions than all other activated carbons. Activated carbon rankings follow the same pattern from most to least adsorption: Darco 12 × 20, Norit GAC 830+, Norit CGran, Sorbonorit B3, GCN 48 (now GCA 48), over both temperatures of 25 °C and 55 °C, respectively. Temperature made a slight difference in % protein and % odor/color adsorptions with 55 °C showing higher amounts than at 25 °C over all activated carbons, even though this was not statistically tested.

3.2. Results with molecular sieves

No transformation of data for percent of protein adsorbed was needed, whereas, the variance for data for percent of odor adsorbed

Table 1
Effect of temperature on the adsorption of zein protein and its odor/color components by activated carbons.

Adsorbent at 25 (°C)	% Protein adsorbed mean (±SD)			% Odor/color adsorbed mean (±SD)		
Sorbonorit B3	52.2	(2.3)	c[a]	85.3	(4.6)	b
GCN 48	37.4	(4.1)	d	72.0	(7.4)	c
NoritGAC 830+	71.4	(2.7)	b	95.5	(1.6)	a
Norit C Gran	57.2	(0.7)	c	92.1	(0.2)	ab
Darco 12 × 20	82.3	(9.9)	a	97.7	(0.6)	a
At 55 (°C)						
Sorbonorit B3	55.6	(0.5)	d	92.4	(0.4)	d
GCN 48	39.3	(0.9)	e	80.8	(0.3)	e
NoritGAC 830+	78.8	(1.5)	b	97.8	(0.5)	b
Norit C Gran	59.1	(0.8)	c	94.1	(0.3)	c
Darco 12 × 20	95.2	(0.8)	a	99.7	(0.1)	a

[a] Sieve means (±SD) within a protein temperature or odor-temperature combination followed by the same letter are not significantly different based on difference of least square means at $p \leq 0.05$.

Table 2
Effect of temperature on the adsorption of zein protein and its odor/color components by molecular sieves.

Adsorbent	% Protein adsorbed mean (±SD)			% Odor/color adsorbed mean (±SD)		
Zeobest	21.40	(3.82)	b[a]	38.52	(5.38)	c
Zeolite 3A	15.65	(4.96)	c	38.17	(7.33)	c
Zeolite 4A	19.55	(3.07)	bc	42.77	(4.31)	b
Zeolite 5A	27.05	(2.22)	a	48.22	(3.78)	a
Zeolite 13X	22.22	(4.39)	ab	47.52	(6.5)	a
Temperature(°C)						
25	20.59	(5.12)	a	38.64	(5.58)	b[b]
55	21.75	(5.35)	a	47.43	(4.78)	a

[a] Sieve means (±SD) within a column followed by the same letter are not significantly different based on differences of least square means at $p \leq 0.05$.
[b] Temperature means (±SD) within a column followed by the same letter are not significantly different based on *F*-test statistic from ANOVA.

was stabilized using a (% odor)^2 transformation. Significant differences in mean % protein and % odor adsorptions for the different molecular sieves were found from the ANOVAs ($p = 0.0022$ and $p < 0.0001$, respectively), along with significant differences in % odor adsorption at the two temperatures ($p < 0.0001$).

From data in Table 2, it is apparent that the native Zeobest sieve has a medium amount of % protein adsorption with greater amounts than 3A, smaller amounts than 5A, and equal amounts to 4A and 13X. The Zeobest sieve has significantly less % odor adsorption than all other sieves but 3A. Zeolite 5A with Ca^{2+} ion showed significantly higher adsorption of % protein and % odor/color adsorption than zeolite 3A with K^+ and zeolite 4A with Na^+ ions, respectively, both with smaller pore sizes. Zeolite 13X with a different crystal structure tended to show less % protein adsorbance and about the same % odor/color absorbance when compared with zeolite 5A. Overall, zeolite 5A consistently gave the most reproducible results with the lowest standard deviations than all other sieves evaluated.

4. Conclusion

Processing zein aqueous ethanol solutions at 55 °C enhanced the adsorptions of the off-odor and color components of zein relative to the adsorption of protein for both the activated carbon media and zeolites resins. This adsorption behavior at 55 °C may result from a combination of events involving solvent environment, viscosity changes, as well as changes in the structure of the zein molecule. Cabra et al. (2008) observed changes in the secondary structure of a purified Z19 α-zein in aqueous ethanol upon heating this protein solution from 25 to 80 °C. Analyses of these solutions by far UV–CD spectroscopy showed diminished helical content and increased random coil formation when those solutions were heated between 50 and 60 °C. Momany et al. (2006) proposed a model for zein, based on molecular dynamics simulations where three lutein molecules fit into the core of the three triad helical segments helping to stabilize the configuration. Conceivably, the loss of the α-helix structure upon heating as observed by Cabra et al. (2008) would release that xanthophyll, thereby, making it more available for adsorption by the activated carbons and zeolites. The change in secondary structure of zein would also have the tendency to reduce the protein–putrescine derivative interactions. Because zein in aqueous ethanol aggregates upon heating (Cabra et al., 2008), the protein aggregates would have a tendency to adsorb to the surface of the activated carbon and zeolite media. Adherence of the aggregated zein may diminish recovery of the purified zein.

Based on the findings in this investigation, we selected zeolite 5A and GCN 48 as column media for our patent-pending process (Sessa, 2007) that involves a multi-columnar filtration. The economics of that process have yet to be determined. The cost of the activated carbon GCA 48 (formerly GCN 48) is $3.83/lb in 55 lb lots. Cost of molecular sieve zeolite 5A is $18.59/lb for 5 kg batches. Because of the "open" pore structure of the zeolite 5A, regeneration of that resin can be readily accomplished. Native zeolite, such as clinoptilolite, though considerably cheaper than synthetic zeolite, may contain heavy metal contaminants that would preclude its use in food grade zein products.

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